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The use of quantitative fetal fibronectin for the prediction of preterm birth in women with exposed fetal membranes undergoing emergency cervical cerclage

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Conception and design of the study: NS, AS, MC; data acquisition: NS, MH; analysis and interpretation NS; drafting and editing of manuscript: NS, MH, AS, MC; recruitment and collection of samples: NS, AS, MC

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Conflicts of interest
Professor Shennan is CI on a Hologic-funded technical study on the use of fetal fibronectin (paid to institution). Dr Suff and Dr Chandiramani received financial assistance for expenses for educational talks on Preterm Birth from Hologic, USA.

Introduction

Globally, over 10% of pregnancies result in preterm delivery[1]. Preterm birth is the leading cause of mortality among children under 5 years old, and children who survive preterm delivery are at increased risk of long term morbidity including neurodevelopmental delay and chronic lung disease[2,3]. Several interventions have been shown to increase prognosis in preterm labour, such as appropriate administration of antenatal corticosteroids[4]. According to NICE guidelines, in women who are found to have exposed membranes between 16 and 27+6 weeks gestation insertion of an emergency cervical cerclage can be considered to reduce the risk of preterm delivery and to prolong gestation to delivery[5]. Insertion of an emergency cervical cerclage has been found to delay delivery by approximately 5 weeks[6,7]. However, emergency cervical cerclage is associated with maternal risks including an increased chance of miscarriage, chorioamnionitis and subsequent need for delivery by Caesarean Section[8]. It has previously been shown that the overall gestational age at delivery, suture insertion to delivery interval, and neonatal outcome for those cases with visible membranes at the time of cerclage insertion were considerably worse than for those women with no visible membranes (median gestational age at delivery, 24^{+2} vs 37^{+5} weeks gestation; median suture insertion to delivery interval, 19 vs 117 days; fetal survival rates, 50% vs 91%)[9].

Fetal fibronectin (fFN) is an extracellular matrix glycoprotein located between the decidua and the trophoblast. Disruption of this interface results in release of fFN into the cervicovaginal fluid. Our group has previously demonstrated the predictive value of quantitative fFN measurement in women presenting with symptoms of preterm labour, with added risk discrimination over and above the traditional qualitative test, as well as in asymptomatic women at high risk of preterm birth[10]. Furthermore, we have shown that even in women with exposed membranes, a group traditionally thought to be at universally high risk of preterm birth, the risk of preterm birth still correlates with qfFN concentration in the cervicovaginal fluid[11].

The purpose of this study is to determine the predictive value of quantitative fFN for preterm delivery among women with exposed membranes undergoing emergency cervical cerclage insertion.

Material and Methods

This is a retrospective observational study performed between 2015 and 2018 at St Thomas' Hospital, London, a tertiary level Obstetrics unit.

The study had ethical approval under the Preterm Birth Clinical Network Database (Research Ethics Committee (REC) Reference 16/ES/0093)[12]. Women were recruited to the study if they had singleton pregnancies, and were considered high-risk for preterm birth (one or more of: previous sPTB <37 weeks' gestation; previous second-trimester miscarriage ≥16 weeks; previous invasive cervical surgery; uterine abnormality; incidental finding of a short cervix <15mm). Women were included in the analysis at the time they had exposed membranes noted at speculum or during cervical cerclage insertion (with any degree of cervical dilatation), if they were between 18 and 24 weeks of gestation, were not contracting, and underwent quantitative fFN concentration measurements within 24 hours prior to emergency cerclage insertion (Hologic Rapid fFN 10Q). fFN testing was performed prior to ultrasound scan or digital examination using previously described methods, in line with the manufacturer's guidelines. Briefly, during speculum examination, a swab was inserted into the posterior fornix and rotated for 10 seconds until saturated, avoiding the cervix and fetal membranes. A single aliquot (200 µl) of the sample was analysed with the conventional qualitative analyser. Women with blood stained CVF samples, sexual intercourse in the previous 24 h, or suspected/confirmed rupture of membranes were excluded from the analysis, due to known interference with fFN measurement.

The primary outcome for analysis was spontaneous PTB within 28 days from testing (as the median gestational age of cerclage insertion is 20 weeks gestation with an aim to prolong gestation to fetal viability at 23-24 weeks gestation) with

additional outcomes of spontaneous PTB within 14 days and prior to 28, 34 and 37 weeks of gestation as well as latency from cerclage insertion to delivery. All statistical analyses were performed with GraphPad Prism software version 8.0. $P<0.05$ was considered statistically significant. Previously established thresholds of qfFN (10, 50, 200, 500 ng/ml) were used to determine sensitivity, specificity, positive and negative predictive values, and relative risk[13]. This study is reported according to the Standards for the Reporting of Diagnostic accuracy studies (STARD) guidelines.

Results

35 women met the inclusion criteria. The demographic and clinical characteristics of the 35 women eligible for analysis are described in Table 1. 17% had a history of spontaneous preterm birth and 46% had a history of second trimester miscarriage. One woman (3%) had a previous history of cervical surgery. Median gestational age at insertion of cervical cerclage was 20+2 weeks (LQ-UQ; 18+6 - 21+2 weeks). Median gestational age at delivery was 29+2 weeks (23+2 - 38+3 weeks) (Table 1).

Table 2 describes the distribution of women in each qfFN category and the rates of sPTB in each group. In total, 12 women (34%) delivered within 28 days of cervical cerclage insertion and 21 women (62%) delivered before 37 weeks gestation (Table 2). 60% of women with an fFN level above 500ng/mL delivered within 28 days of cerclage insertion, and they all delivered before 37 weeks gestation. No women with fFN levels below 10ng/mL delivered within 14 or 28 days and 75% of them delivered at term.

The median time from cervical cerclage insertion to delivery was 65.5 days (17 - 126.5 days) (Table 1). Spearman's rank-order correlation was significant between fFN concentration at time of cerclage and number of days to delivery ($r_s=-0.52$, $p=0.0016$), which appeared to follow a linear trend (Figure 1). Mann–Whitney U analysis demonstrated a significant difference in the distribution of qfFN concentrations in women who delivered preterm compared to those who

did not, both within 28 days from the cerclage (p=0.0048) and those less than 37 weeks gestation (p=0.006).

Predictive statistics for sPTB within 28 days of cerclage insertion and prior to 37 weeks of gestation using qfFN are described in Table 3 and 4, respectively. For delivery within 28 days, fFN ≥ 200 ng/mL had a 72% sensitivity and 63% specificity (Table 3). For delivery before 37 weeks, fFN ≥ 10 ng/mL had 95% sensitivity, while fFN ≥ 500 ng/mL had 100% specificity (Table 4). There is a significant difference in time to delivery based on a qfFN threshold of >200 ng/mL; woman with a fFN value of 0-199 ng/ml had a median time to delivery of 124 days (range 68-140 days), whereas women with a fFN >200ng/ml had a median time to delivery of 29 days (range 12-75) (p = 0.0014). This is represented in a Kaplan Meier survival curve comparing the gestations at delivery between women with fFN levels<200ng/mL and those with fFN levels<200ng/mL prior to cerclage insertion.

Discussion

This study demonstrates the prognostic value of pre-cerclage qfFN levels to predict spontaneous PTB in women with exposed membranes. Our results show that the risk of preterm birth correlates with qfFN concentration in CVF even in women with exposed membranes, a group generally considered to be at extremely high risk of preterm birth. It is likely the leakage of fFN represents an active inflammatory process, related to risk of delivery following chorio-decidual disruption, rather than a mechanism solely related to close proximity of the exposed membranes to the vagina.

There is a significant negative correlation between pre-cervical cerclage fFN and risk of preterm delivery. Women with a pre-cerclage fFN of 0-199ng/ml are significantly less likely to have preterm delivery than women with an fFN ≥200ng/ml. Women with an fFN of >500ng/ml were shown to be at extremely high risk of preterm delivery.

Women with exposed but with low concentrations (<200ng/mL) of cervicovaginal fFN levels have a low risk of subsequent delivery before 37 weeks after cerclage insertion compared with women with higher concentrations (>200ng/mL) of qfFN (RR = 2.8, 1.5-6.3). In this cohort, no women with qfFN <10 ng/mL delivered within 28 days, whilst 40% of those with qfFN concentrations ≥200 ng/mL and 60% women with qfFN ≥500 ng/mL delivered within this time frame. Furthermore, the positive prediction of qfFN at thresholds of 200 ng/ml was superior to the traditionally used qualitative test (threshold of 50 ng/ml), whilst maintaining good negative prediction. However, exposed fetal membranes and the use of emergency cerclage remain rare events and as such highly powered statistical analysis has not been possible in this study.

To our knowledge, this is the first study looking at the prognostic value of fFN among women requiring emergency cerclage for exposed membranes. Our group has previously demonstrated the prognostic value of fFN among women with risk factors for preterm labour but without exposed membranes[10] and more recently with exposed membranes[11].

Currently the routine use of fetal fibronectin tests in women with exposed fetal membranes is not often performed, nor is it recommended[14]. However, this study suggests a role for the test in determining risk of preterm delivery and also the likelihood of reaching viability amongst women with exposed fetal membranes who are suitable for emergency cerclage. Given the high failure rate of this type of cerclage and the associated maternal septic morbidity that can be serious and potentially require ITU admission and result in end-organ damage, having this test to aid in clinical decision making seems prudent. It may support the clinician's decision not to intervene in certain situations and accept a poor outcome, in order to do no harm. Furthermore, quantitative fFN in this group could have wider implications on other interventions for the management of threatened preterm delivery such as antenatal steroids and magnesium sulphate use, as well as transfer to tertiary neonatal units after 24 weeks' gestation.

Nonetheless, larger studies of quantitative fFN in this group of patients are required to confirm its accuracy and also to confirm test thresholds with the highest positive predictive values. Given the rarity of cervical cerclage for exposed fetal membranes, this would likely need to be done across multiple sites to improve homogeneity and to provide sufficient statistical power. Future work could also determine whether the insertion of an emergency cervical cerclage should be guided by the result of the pre-cerclage fetal fibronectin level.

Conclusion

The use of cervicovaginal quantitative fFN levels in women presenting with exposed membranes between 18^{+0}-23^{+6} weeks gestation with no contraindications to cervical cerclage is beneficial in providing information on the risk of preterm delivery. Given the serious maternal and fetal risks associated with emergency cerclage surgery, qfFN could be used to counsel these patients at high risk of preterm delivery as well as potentially assisting in the clinical decision to insert a cerclage.

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